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VARIATION IN DIETARY FIBRE CONTENT, STARCH QUALITY AND PASTING PROPERTIES OF OAT FLOURS - UNDERSTANDING THE EFFECT OF OAT MILLING PROCESS

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ABSTRACTS

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Tiivistelmä – Referat – Abstract

Oats (*Avena sativa* L.) are increasing their popularity as a food ingredient since they have excellent nutritional value and great applicability in various food categories. Oats have been mainly used as feed and their food processing properties have not been studied as extensively as of the other cereal grains. Previous studies indicate that oat milling process can cause changes in the carbohydrate quality and properties of raw material. The aim of this Master's thesis was to understand the impact of oat milling process and dry fractionation on oat ingredient characteristics in 10 samples representing Finnish oat varieties. The selected oat ingredients were non-heated oat groats, oat flour produced by industrial scale milling process and starch-rich fraction obtained from the oat flour by air classification aiming at bran-endosperm separation. The hypothesis was that the oat milling process as well as fractionation affect the physicochemical properties of oat ingredients and that the different oat raw materials may differ regarding their carbohydrate properties and processing behaviour. Dietary fibre, damaged starch and amylose contents of the oat ingredients were analysed. To understand the physicochemical properties of different oat ingredients, the pasting properties were measured with Rapid Visco Analyser (RVA). As expected, oat milling affected both quality and physicochemical properties of the oat raw materials. Milling caused a significant increase in the damaged starch content and caused changes in almost all pasting parameters. Furthermore, sample-dependent behaviour was observed in pasting properties. The oat samples showed differing behaviour during dry fractionation. The pasting properties of the air classified starch-rich fractions were different from the raw material oat flour. These results confirm that different oat raw materials exhibit different behaviour during oat milling process and that the milling process has a significant impact on physicochemical characteristics of oats.

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Oats (*Avena sativa* L.), oat milling, air-classification, dietary fibre, starch, pasting properties

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Kauran (*Avena sativa* L.) suosio elintarvikekäytössä on kasvanut viime vuosikymmenen ajan sen erinomaisen ravintoarvon ansiosta. Kauraa on perinteisesti käytetty rehuna, minkä vuoksi sen elintarvikeominaisuuksia ei ole tutkittu yhtä kattavasti kuin muiden ruokaviljojen. Aiemman kirjallisuuden perusteella kauran prosessointi voi vaikuttaa kauran hiilihydraattien laatuun ja ominaisuuksiin. Tämän maisterintutkielman tavoitteena oli selvittää, miten kauran jauhatus ja kuivafraktiointi vaikuttavat 10 suomalaisesta kauralajikkeesta valmistettujen myllytuotteiden ominaisuuksiin. Tutkitut raaka-aineet olivat kuumentamattomat, kuoritut kauranjyvät, kauramyllyllä jauhettu kaurajauho ja kaurajauhosta ilmaluokituksella tuotettu tärkkelyspitoinen jae. Hypoteesina oli, että sekä jauhatuksella että ilmaluokittelulla on vaikutusta kauranäytteiden fysikaaliskemiallisiin ominaisuuksiin. Oletuksena oli myös, että eri raaka-aineet eroavat toisistaan prosessoitavuuden sekä hiilihydraattikoostumuksen ja laadun suhteen. Raaka-aineista määritettiin ravintokuidun ja vaurioituneen tärkkelyksen pitoisuus sekä amyloosin osuus tärkkelyksestä. Fysikaaliskemiallisten ominaisuuksien ymmärtämiseksi näytteiden liisteröitymisominaisuudet mitattiin Rapid Visco Analyser-laitteella (RVA). Kauran teollisen jauhatusprosessin todettiin vaikuttavan sekä kauran hiilihydraattien laatuun että fysikaaliskemiallisiin ominaisuuksiin. Jauhatusprosessi lisäsi merkittävästi vaurioituneen tärkkelyksen määrää näytteissä ja aiheutti muutoksia lähes kaikissa liisteröitymisparametreissa. Eri näytteiden välillä huomattiin myös näytekohtaisia eroja. Kymmenen kauranäytteen luokittuvuudessa oli eroja, ja luokiteltu tärkkelyspitoinen jae erosi liisteröitymisominaisuuksiltaan alkuperäisestä kaurajauhosta. Tämän tutkielman tulokset osoittavat, että erilaiset kaurat eroavat jauhatusominaisuuksiltaan, ja että kauran jauhatusprosessilla on merkittävä vaikutus kauran fysikaaliskemiallisiin ominaisuuksiin.

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PREFACE

This Master's thesis was part of the OatHow project funded by Business Finland. The experimental work and writing were conducted at VTT Technical Research Centre of Finland Ltd (VTT) from March 2019 to early April 2020. The Professor in charge was Vieno Piironen from University of Helsinki and supervisors were Ulla Holopainen-Mantila (VTT) and Pia Silventoinen (VTT).

I want to thank my supervisors for their invaluable support and guidance throughout this thesis project. I am very grateful for both, Ulla Holopainen-Mantila and Pia Silventoinen for broadening my perspective regarding food science and helping me to think more out of the box. I wish to express my gratitude to Professor Vieno Piironen for the excellent guidance and advice during this thesis work as well as during my whole studies. I am grateful to Martina Lille for sharing her expertise in RVA measurement with me, and to Atte Mikkelson for executing the HPLC measurements and interpreting them. I want to thank Eero Mattila, Leila Kostamo, Tarja Wikström and Riitta Pasanen for their assistance during my experimental work and for teaching me all the analytical methods used in this thesis. Great thanks also to Tytti Salminen and Mihray Mijit for their assistance during my experimental work.

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Iina Jokinen

LIST OF ABBREVIATIONS

BDV	Breakdown viscosity
Con A	Concanavalin A
DE	Diatomaceous earth
DMSO	Dimethyl sulphoxide
FV	Final viscosity
GOPOD	Glucose oxidase-peroxidase reagent
IDF	Water-insoluble dietary fibre
PT	Pasting temperature
PV	Peak viscosity
SBV	Setback viscosity
SDFP	Water-soluble dietary fibre that precipitates in 78 % ethanol
SDFS	Water-soluble dietary fibre that stays soluble in the presence of 78 % ethanol
SSE	Starch separation efficiency
TDF	Total dietary fibre
TTPV	Time to reach peak viscosity
TV	Trough viscosity

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1 INTRODUCTION

Common oats (*Avena sativa* L.) have been traditionally used as feed, but during recent decades oats have been proven to have also a great potential as a food ingredient (Butt et al. 2008; Webster 2011; Arendt and Zannini 2013; Kouřimská et al. 2018). Oats are well adapted to different soil conditions and temperate climates and have good nutritional composition (Peterson et al. 1975; Butt et al. 2008; Biel et al. 2009; Kouřimská et al. 2018). Oats have high lipid content and they are a good source of dietary fibre, proteins, minerals and other bioactive compounds. Dietary fibre component β-glucan has especially increased the interest towards oats as a food raw material due to its properties related to lowering blood cholesterol (FDA 1997; Wood 2011). The worldwide production of oats has been slowly increasing during the recent years with current production of approximately 26 million tonnes annually (FAO 2019).

Oat kernel has similar structure to other cereal grains consisting of hull, pericarp, testa, nucellus, aleurone layer, subaleurone layer, endosperm and embryo (Miller and Fulcher 2011; Arendt and Zannini 2013). However, the chemical composition of oats is unique compared to more commonly used wheat, maize, rice and barley (Butt et al. 2008). The most significant difference is that oats have higher lipid content than other cereal grains and the lipids are distributed more evenly in the kernel. When considering oats as food, the composition is typically reported for dehulled oat grains called as groats, after removal of indigestible hull (Butt et al. 2008; Gulvady et al. 2014). The chemical composition of groat varies depending on the growing location, growing conditions and variety (Mut et al. 2018). Oat groat is composed of 40-68% starch, 12-25% protein, 4-16% lipids, 10-19% dietary fibre and 1.9-3.5% ash in dry matter basis (Lásztity 1998; Zhou et al. 1998; Lapveteläinen et al. 2001; Hüttner et al. 2010; Kouřimská et al. 2018).

Starch and dietary fibre, have a prominent role in both food processing and human nutrition (Huber and Bemiller 2017). Starch is the main energy source in human nutrition whereas dietary fibre is not digested in human small intestine and has more impact on promoting normal laxation and bowel movement as well as decreasing the cholesterol intake from diet (EFSA 2010; Huber and Bemiller 2017). Both starch and dietary fibre affect the physicochemical and technological properties of oats, and therefore understanding their role in oat processing is important.

The dietary fibre is defined by the CODEX Alimentarus (FAO and WHO 2017) as "Carbohydrate polymers with ten or more monomeric units, which are not hydrolysed by the

endogenous enzymes in the small intestine of humans and belong to the following categories: 1) edible carbohydrate polymers naturally occurring in the food as consumed, 2) carbohydrate polymers, which have been obtained from food raw material by physical, enzymatic or chemical means and which have been shown to have a physiological effect of benefit to health as demonstrated by generally accepted scientific evidence to competent authorities and 3) synthetic carbohydrate polymers which have been shown to have a physiological effect of benefit to health as demonstrated by generally accepted scientific evidence to competent authorities.". The European commission (EC) has defined in regulation 1169/2011 the dietary fibre according to CODEX definition, but has included also the carbohydrates with three to 9 monomeric units to the definition (EC 2011).

Several analytical methods have been developed for analysing total dietary fibre (TDF) content of foods from which AOAC methods 2009.01 and 2011.25 measure the TDF content according to the CODEX definition (McCleary et al. 2013). Different TDF methods use different terminology, and in this thesis, dietary fibre components are referred to as water insoluble dietary fibre (IDF) and water soluble dietary fibre (SDF) according to nomenclature used in AOAC method 2011.25 (McCleary et al. 2012). In this methodology, SDF includes water-soluble dietary fibre that precipitates in 78% (v/v) ethanol (SDFP) and water-soluble dietary fibre that stays soluble in 78% (v/v) ethanol (SDFS).

Oat groats contain 10-12% dietary fibre in dry matter basis (Manthey et al. 1999; Rainakari et al. 2016). TDF content is 14-19% in oat bran and 4-19% in oat flour (Hüttner et al. 2010; Arendt and Zannini 2013; Rainakari et al. 2016; Aprodu and Banu 2017; Fineli 2019). Different proportions of bran layers in commercial flours analysed in the literature explain the large variation in oat flour TDF content. In general, oat flours containing mainly endosperm have lower TDF content. IDF content in oat groats varies between 6.0-8.3 % (Manthey et al. 1999; Rainakari et al. 2016; Aprodu and Banu 2017). Oat IDF fraction consists of 50% of dietary fibre polysaccharides, mainly β-glucan but also arabinoxylan and rest of the IDF fraction is composed 43% of Klason lignin and 7% of uronic acid (Manthey et al. 1999). SDF content of oat groats varies from 3.9-5.9% in dry matter basis (Manthey et al. 1999; Rainakari et al. 2016). Rainakari et al. (2016) studied the dietary fibre content with AOAC method 2011.25 and reported IDF contents of 6.7-8.1% and SDF contents of 3.9-5.9% in rolled oats in dry matter basis. The SDFP content in rolled oats varied between 3.7-5.6% and SDFS content between 0.2-0.5% in dry matter basis. Oat SDF is mainly β-glucan (80%), but contains also arabinoxylan and arabinogalactan (Manthey et al. 1999). Oat β-

glucan is a linear polysaccharide consisting of approximately 30% of β-(1→3)-linked and 70% of β-(1→4)-linked β-D-glucopyranosyl units, as reviewed by Liu et al. (2010).

Starch is one of the most abundant biopolymers in food ingredients (Vamadevan and Liu 2016; Huber and Bemiller 2017). It is the main energy storage form in plants and exists as starch granules composed of two polysaccharides, amylose and amylopectin. Both polysaccharides are composed of α-D-glucopyranosyl units; amylose is essentially a linear polymer with some branches whereas amylopectin is highly branched. Due to the characteristic properties of starch, gelatinisation, pasting and retrogradation, it can be widely utilized in several kind of food applications (Swinkels 1985; Whistler and BeMiller 2009; Huber and Bemiller 2017).

Starch content of the oat groat is typically 50-60% in dry matter basis, although there can be great variation in oat starch content depending on the growing location, oat variety and the proportions of other macromolecules (Doehlert and Moore 1997; Zhou et al. 1998; Shewry et al. 2008; Arendt and Zannini 2013). Oat starch granules are either compound granules composed of several sub-units or individual single starch granules (Stevenson et al. 2007; Saccomanno et al. 2017). Single granule and sub-unit granule size varies between 3-12 µm and compound granule size between 60-80 µm (Gudmundsson and Eliasson 1989; Hoover and Vasanthan 1992; Hoover and Senanayake 1996; Hoover et al. 2003; Stevenson et al. 2007; Saccomanno et al. 2017). Lipid content of oat starch is 0.9-2.5%, which is higher than in other common starches. Oat starch amylose content varies between 19.6-31.6% in dry matter basis, but also even higher values have been reported (Morrison et al. 1984; Hoover et al. 2003; Hüttner et al. 2010; Ziegler et al. 2018).

One of the important physicochemical properties affecting the food applicability of starch is pasting behaviour (Kasturi and Bordenave 2014; Huber and Bemiller 2017; Balet et al. 2019). In pasting, starch granules swell in the presence of heat and water resulting in the leaching of soluble granule components and eventually granule disruption as reviewed by Kasturi and Bordenave (2014) and Huber and Bemiller (2017). The released granule components form a viscous paste. When the paste is cooled, the polymers re-associate and viscosity further increases. This phenomenon is referred to as retrogradation. Starch pasting properties depend on the starch composition and structure as well as presence of other components.

Starch pasting properties can be measured with several instruments from which Rapid Visco Analyser (RVA) is commonly used due to its repeatability, reliability and versatility (Balet

et al. 2019). RVA measures the change in the paste viscosity under a shear as a function of time and temperature (Zhou et al. 1998; Crosbie and Ross 2007; Balet et al. 2019). A typical RVA profile includes initial stage, heating stage, holding stage, cooling stage and final holding stage (Figure 1.) (Zhou et al. 1998; Balet et al. 2019). In initial stage, the sample is mixed with water or solvent and in the heating stage the sample is heated, first gelatinization, then pasting occurs, and viscosity increases. In the first holding stage the temperature is kept constant and viscosity decreases, while in the cooling stage the sample is cooled, and viscosity begins to increase again due to occurring retrogradation. In the second holding stage, the temperature is kept constant and sample reaches viscosity plateau.

Important parameters measured in RVA are pasting temperature (PT), peak viscosity (PV), time to peak viscosity (TTPV), breakdown viscosity (BDV), trough viscosity (TV) and setback (SBV) (Figure 1.) (Balet et al. 2019). PT is expressed as the temperature in which the viscosity of the sample starts to increase and pasting occurs. PV presents the highest viscosity that the sample reaches during heating stage and TTPV is the time taken to reach the PV. BDV viscosity is followed by peak viscosity during first holding stage when viscosity decreases due to melting of the crystalline regions of the starch granule and is measured as the difference between peak viscosity and trough viscosity. Trough viscosity represents the lowest viscosity reached during first holding stage. Final viscosity is recorded as the viscosity of the sample in the end of the RVA measurement. Setback viscosity tells about the viscosity difference between trough and final viscosity values and it is related to the tendency of the starch sample to retrograde i.e. higher SB values are related to higher tendency of the sample to retrograde (Balet et al. 2019).

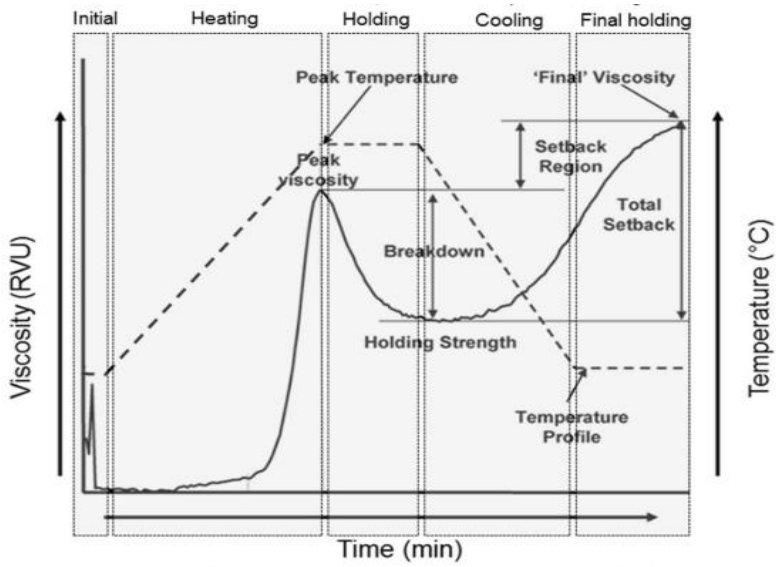

Figure 1. A typical RVA pasting curve of a cereal grain when using the standard method profile (Balet et al. 2019).

Both oat starch and oat flour pasting properties have been measured with RVA. High pasting temperatures resulting from high intrinsic lipid content are characteristic of native oat starch (Hoover and Vasanthan 1992; Hoover et al. 2003). In whole oat flour, other components such as protein and dietary fibre affect the pasting properties in addition to starch (Liu et al. 2010; Choi et al. 2012). In the study by Liu et al. (2010) it was shown that β-glucan has significant impact on oat flour pasting properties. They also observed that interactions between β-glucan and starch or protein have effect on pasting properties. β-glucan content exhibits positive correlation with peak, through and final viscosity values (Choi et al. 2012).

Oat milling and processing can affect the carbohydrate quality and properties of oats (Zhou et al. 1998; X. Hu et al. 2010; Nguyen et al. 2019). Oat milling has some special features when compared to other cereal grains (Girardet and Webster 2011). As previously noted, oats have high lipid content, which affects their processing behaviour. In addition, oats have high activity of endogenous enzymes such as lipase and peroxygenase, which readily cause lipid degradation in oat grains the moment the cells are disrupted(Yang et al. 2017). To tackle this issue, a heat treatment by steaming, also known as kilning, has been developed to inactivate endogenous enzymes that can induce lipid degradation in oat flours (Girardet and Webster 2011; Arendt and Zannini 2013). Heat treatment has been reported to affect the physicochemical properties of starch and has been shown to have an effect on oat flour and oat starch quality and physicochemical properties (Zhou M. et al. 1999; Hoover 2010; X. Hu et al. 2010; Ziegler et al. 2018; Nguyen et al. 2019). In addition, the mechanical forces present in the milling process can cause damage to starch granules (Morgan and Williams 1995; Boyaci et al. 2004; Assatory et al. 2019).

Oat ingredients are increasingly used in both traditional bakery products such as breads and biscuits as well as in more novel products such as yoghurt-type products and oat-based drinks. Therefore, there is a need to understand better the behaviour of oats during milling process and the properties of the different oat ingredients. Oats are delivered to the mills as a mixture of different oat varieties and therefore the literature regarding the commercial oat ingredients does not provide understanding on the properties of oat ingredients produced only from a single variety (Lapveteläinen et al. 2001; Girardet and Webster 2011). After the milling process, dry fractionation methods, such as air-classification, can be used to separate the oat flour into the endosperm and bran fractions (Sibakov et al. 2012; Assatory et al. 2019). Understanding the effect of the milling and dry fractionation processes on the oat ingredient characteristics can improve the applicability of oat ingredients in food processes.

The aim of this Master's thesis was to understand the impact of dietary fibre content and starch quality on oat ingredient characteristics. More specifically, the goal was to study the impact of oat milling process on oat starch quality and on physico-chemical properties of oat ingredients measured with Rapid Visco Analyser (RVA) as well as their impact on and behaviour in dry fractionation. The selected oat ingredients were obtained from samples of 10 Finnish oat varieties 1) as non-heat-treated oat groats, 2) after industrial scale dehulling, steaming, flaking and milling and 3) after fractionation of the industrial scale processed sample by air classification. The hypothesis was that the milling scale and steaming as well as fractionation affect physicochemical properties of oat ingredients and that the different oat raw materials may differ regarding their processing behaviour and carbohydrate properties. Results from the different raw materials and fractions produced from them by air classification provided information about impact of both starch properties (e.g. damaged starch and amylose content) and dietary fibre content on processing behaviour as well as on physicochemical properties.

2 EXPERIMENTAL RESEARCH

This Master's thesis is part of the OatHow (Novel indicators and technologies for oat quality and applicability, 2019-2020) research project funded by Business Finland. The project aims to produce new information about oat-based raw materials for development of novel oat innovations. The objective is also to gain more knowledge on how the chemical composition of oats affects the processability and food applicability of different oat samples. Four research partners, University of Helsinki, University of Turku, VTT Technical Research Centre of Finland Ltd. and Natural Resources Institute Finland (Luke), conduct the project and also thirteen company partners are involved. This thesis is part of work packages 1 and 2 (WP1 and WP2, respectively). WP1 aims to determine the chemical, physical and agronomic properties from the non-heat-treated oat groat samples whereas WP2 aims to study the physicochemical and sensory properties of the oat samples processed in industrial scale including heat treatment typical for oat processing. The samples analysed in this work were non-heat-treated oat groats (G), industrially milled oat flour (NFL) and fine fraction produced by air-classification from the oat flour (NFL-F) (figure 2). Total dietary fibre content was determined only from the oat flours (NFL). Amylose content was determined from the oat flours (NFL) as well as from the corresponding fine fractions produced by air classification (NFL-F). Damaged starch content and pasting properties were determined from all of the samples (G, NFL and NFL-F).

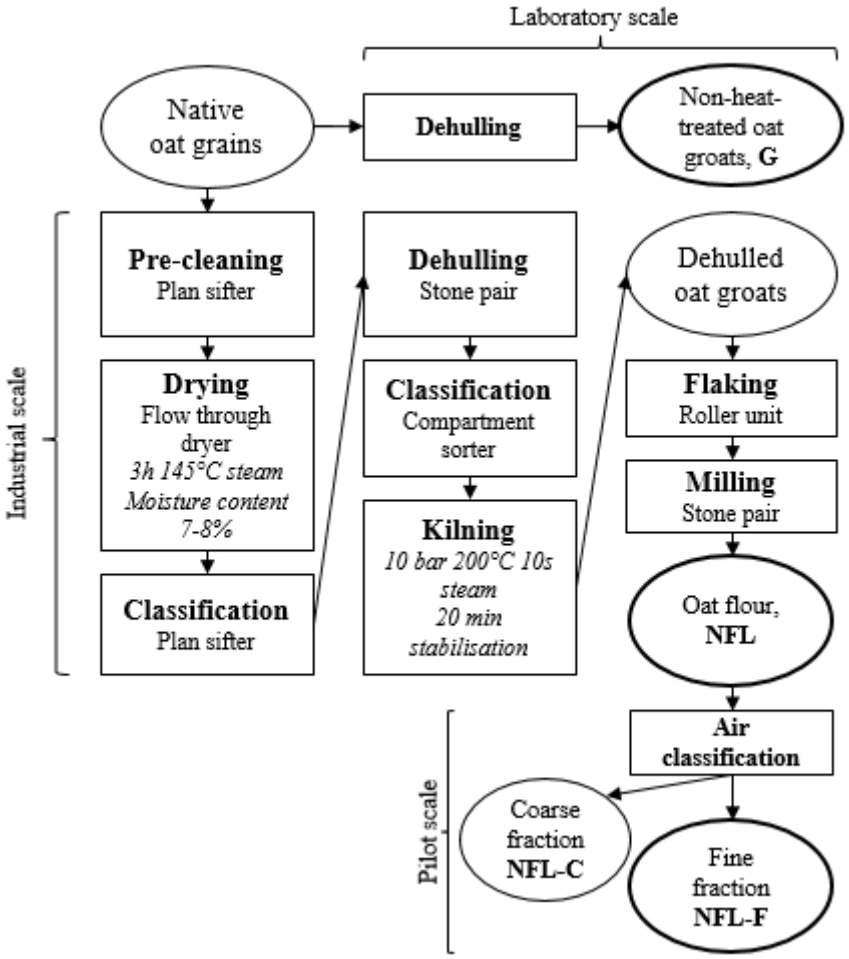

Figure 2. Oat processing steps and different samples analysed in this work. Non-heat-treated oat groat (G) samples were dehulled in laboratory scale, oat flour (NFL) samples were milled in industrial scale at Vääksyn Mylly Oy (Asikkala, Finland) and fine fraction was produced by air classification (NFL-F) in pilot scale from oat flour sample (NFL).

2.1 Materials and methods

2.1.1 Materials

Ten oat varieties (Table 1.) were chosen for this study and were obtained from Boreal Plant Breeding Ltd., Plantanova Oy and Lantmännen Agro Oy. Oat varieties were chosen based on the expectation that they would differ in their composition and physical properties. Two different crop years, namely 2017 and 2018, were represented. Non-heat-treated oat groat samples (G) were stored below -18°C and oat flour samples (NFL) and their air-classified fractions (NFL-F) were stored at +12°C.

Table 1. Oat sample codes, crop years and starch content of non-heat-treated oat groat (G) and oat flour (NFL) samples. Oat variety coding is according to OatHow-project.

Oat variety		Non-heat-treated oat groat samples		Oat flour samples	
Code	Crop year	Code	Starch content (% dm)[1]	Code	Starch content (% dm)[1]
001	2018	G001	63.5	NFL001	61.7
002	2018	G002	61.1	NFL002	59.7
003	2018	G003	67.4	NFL003	65.8
004	2017	G004	72.1	NFL004	67.7
005	2017	G005	70.4	NFL005	65.9
006	2017	G006	74.6	NFL006	71.9
007	2017	G007	73.1	NFL007	69.8
008	2017	G008	75.3	NFL008	70.5
010	2017	G010	71.1	NFL010	69.0
013	2018	G013	60.4	NFL013	58.4

[1]Values obtained from Luke, analysis done during 12/2019-02/2020 according to the method described by Salo and Salmi (1968).

All reagents were of analytical grade and purity and obtained from VWR Chemicals (VWR International, Radnor, PA, USA), Sigma-Aldrich (St. Louis, MI, USA) and Riedel de Haën (Seelze, Germany). Water used in buffer solutions was purified with Milli-Q system (Millipore Corp., Bedford, MA, USA) and water purified with reverse osmosis was used in dietary fibre analysis and in RVA.

2.1.2 Material pre-treatments and processing

Native oat grains were dehulled in laboratory scale in Luke with oat dehuller (Rivakka, Nipere Oy, Finland). Approximately 50 g of dehulled groats were weighed with balance (Precisa 4000C, PAG Oerlikon AB, Switzerland) and ground with ultra-centrifugal mill (Retsch ZM 200, Germany) using 0.5 mm sieve and 12 000 rpm speed. These samples were coded with letter G.

Oat flours were milled at Vääksyn Mylly Oy (Asikkala, Finland) to produce industrial scale processed oat flour (NFL) (Figure 2.). The industrial scale milling included the kilning step to inactivate lipid degrading enzymes. First step of the oat milling process was a pre-cleaning for the whole oat grains with plan shifters in order to remove other grains, seeds, stones and debris. After pre-cleaning, a drying step was performed to obtain a relative seed moisture content of approximately 8%. Oat grains were dried in flow-through dryer using 145°C steam for 3 h. Dried oat grains were classified by size with plan shifter to three different size classes to ensure optimal dehulling and each group was dehulled with stone pair dehuller. The dehulled grains were then kiln dried with steam by convection using 155°C steam for 40 s after which the groats were let to rest for approximately 20 min at approximately 110°C. The approximate final moisture content of the groats was 11%. Kilned oat groats were

processed into oat flakes with a roller unit and the flakes were further milled with stone mill into flours.

Air-classification was performed using a Minisplit Air Classifier (British Rema Manufacturing company Ltd., Chesterfield, UK) with 2500 rpm classifier wheel speed, 220 m^3/h air flow and sample feed rate of approximately 1.7 kg/h. The aim was to obtain coarse fraction representing 20-30% of the original sample weight and fine fraction representing 70-80% of the original sample weight (Sibakov et al. 2012) targeting separation of bran (coarse) and endosperm (fine). In each batch, 700 g of NFL was air classified and the masses of the two fractions were weighed and recorded. All samples were air classified in duplicates and mass yield of each fraction was calculated with equation 1 and the starch separation efficiency (SSE) was calculated with equation 2. (Tyler et al. 1981).

$$Mass\ yield\ (\%\ dm) = \frac{Dry\ weight\ of\ fraction\ (g)}{Dry\ weight\ of\ raw\ material\ (g)} \times 100 \quad (1)$$

$$SSE\ (\%\ dm) = \frac{Dry\ weight\ of\ fraction\ (g) \times starch\ content\ of\ the\ fraction\ (\%)}{Dry\ weight\ of\ raw\ material\ (g) \times Starch\ content\ of\ the\ raw\ material\ (\%)} \times 100 \quad (2)$$

2.1.3 Methods

2.1.3.1 Dietary fibre analysis

For analysing total dietary fibre content of the oat flours in the current study, the method AOAC 2011.25 validated by McCleary et al. (2012) was chosen because in this method the DF content is determined as defined by the Codex Alimentarus. In the current study, the method was conducted with a semi-automated Dietary Fibre Analyser (Figure 3) (ANKOM^{TDF}, Makedon NY, USA), which is a modern replacement for the traditional and slow manual methods. In this method, the analyser performs the laborious filtering steps and therefore shortens the analysis time significantly. The following enzyme solutions and standards of an Integrated total dietary fibre kit (K-INTDF, lot 190224-2, Megazyme, Wicklow, Ireland) were used in dietary fibre analysis: Pancreatic α-amylase (100 000 Ceralpha units/g), amyloglucosidase (3300 units/ml), protease (350 tyrosine units/ml), LC retention time standard (maltodextrins and maltose in 4:1 ratio) and D-sorbitol (dry).

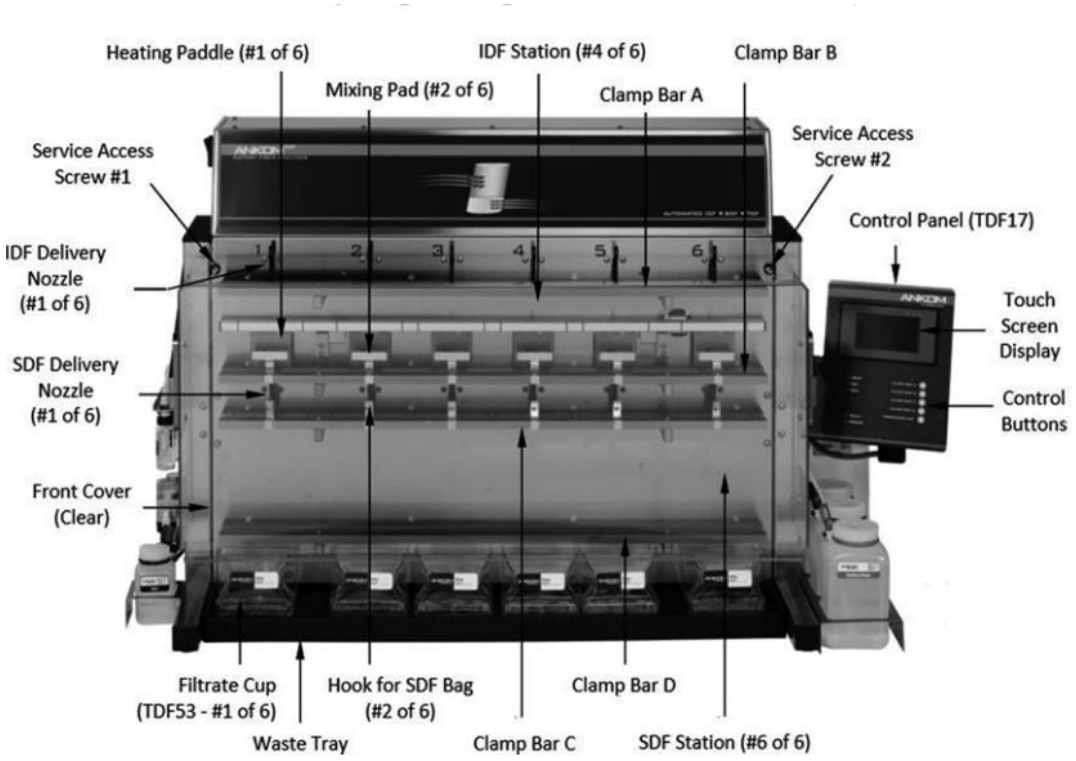

Figure 3. Image of ANKOM^{TDF} Dietary Fiber Analyzer from ANKOM^{TDF} Operator's Manual, ANKOM Technology, Macedon, NY, USA. Used with permission.

The method starts with weighing bags (integrated filter, ANKOM), diatomaceous earth (DE) for both IDF and SDFP bags and the sample. IDF bags were installed below the IDF delivery nozzles (upper) and SDF bags below the SDF delivery nozzles (lower) (figure 3). In IDF bags DE aids in removing the residual sample from the bag after filtration step for protein content determination. In SDF bags it improves the precipitation of SDFP and filtration of SDFS (ANKOM 2016). Then 0.5 ± 0.05 g of flour sample was weighed in duplicate into tared sample tins. Equipment installation started with installation of the SDF filter bags into the equipment and DE was transferred to SDF filter bags using maximum of 3 ml of 78% ethanol. Then IDF bags were installed and samples and DE were transferred into IDF filter bags from tins using max 3 ml of de-ionised water. Digestion with a 2 ml of mixture of α-amylase (2000 Ceralpha units/sample) and amyloglucosidase (136 units/sample) for 16 hours at 37°C was started by selecting a AOAC 2011.25 programme, which automatically added the 50 mM sodium maleate buffer and the α-amylase-amyloglucosidase enzyme solution to the IDF filter bags. At the end of incubation, trizma base was added automatically to the upper filter bag after which the pH was measured manually with pH-meter (pH3310, WTW, Germany) and adjusted with 1 M NaOH to 7.9-8.4 if necessary. Samples were heated to 90°C and incubated for 20 minutes to terminate the enzymatic reaction. Then samples were cooled to 60°C and digested with protease (35 Tyrosine units/ml/sample) for 30

minutes. For termination of the protease digestion, 2 M acetic acid was added automatically and pH was measured manually and adjusted with 1M HCl to pH 4.3 if necessary. After this, one millilitre of an internal D-sorbitol standard (100 mg/ml) was added to each sample.

The sample solution was automatically filtered into SDF bags and IDF fraction remained in the upper bag. IDF bags were automatically rinsed twice with distilled water, then twice with 78% ethanol and twice with 95% ethanol. After rinsing, the IDF (upper) filter bags were manually removed from the equipment and rinsed twice with acetone. After acetone had evaporated the bags were sealed with a heat sealer (Impulse sealer AIE 200-2, AIE, USA) set at value 3.5 and dried in an oven (OP100, LTE Scientific, Oldham, England) at 105°C for 90 minutes. Dried bags were placed in a desiccant pouch, let to cool down, and then weighed. When the SDF fraction had been filtrated to lower filter bags, the SDFP was precipitated for 60 minutes with 95% ethanol. SDFS fraction was then filtrated to glass containers with vacuum assist. The lower bags containing the precipitated SDFP residue were rinsed twice with 78% ethanol and twice with 95% ethanol. These rinsing liquids were filtered into the glass containers as well. After rinsing, the SDF (lower) filter bags containing precipitated SDFP were manually removed from the equipment, rinsed twice with acetone, dried and weighed.

Both IDF and SDFP residue weights were corrected with their protein and ash contents. Protein contents of the IDF and SDFP fractions were determined based on the total nitrogen content analysed with a Kjeldahl autoanalyzer (Foss Tecator Digestion System, Kjeltec2300 analyser unit, Höganäs, Sweden). Samples were removed from the filter bags by cutting carefully the bags open and transferring samples from inside of the bags into Kjeldahl tubes. The protein content was calculated by using conversion factor 6.25. To obtain the ash content, the whole filter bags containing the IDF and SDFP residues were combusted in tared crucibles using furnace (Model P300 N11/HR, Nabertherm, Lilienthal, Germany). The combustion program included a temperature rise from ambient to 103°C during 3 hours, hold in 103°C for 4 hours, rise to 550°C during 9 hours, and hold in 550°C for 7 hours. After the crucibles had cooled sufficiently they were moved into a desiccator, cooled to ambient temperature and weighed.

SDFS content of the remaining filtrate from one of the sample duplicates was determined with high a performance liquid chromatography (HPLC) method. Approximately half of the filtrate was transferred into a 500 ml evaporator flask and evaporated to dryness under vacuum using a rotary evaporator (Hei-VAP Advantage, Heidolph, Germany) at 60°C. The remaining sample was carefully redissolved in 5 ml of deionised water. To deionise the

sample, 2 ml of this solution was transferred on top of the Bio-Rad columns packed with Amberlite FPA 53 (OH^-) and Ambersep 200 (H^+) (Megazyme, Wicklow, Ireland) mixture containing 4 g of each resins. After the sample had entered the resin, 2 ml of deionised water was added to the resin and allowed to percolate in. After this, 20 ml of deionised water was added on top of the column and eluted at the rate of 1 ml/min. The eluate was transferred to a 250 ml evaporator flask, and evaporated into dryness under vacuum at 60°C. The remaining sample was redissolved in 2 ml deionised water, transferred into 3 ml syringe and filtered through a 0.45 µm HPLC grade filter.

The HPLC equipment consisted of a separation module (Waters Alliance 2695, Waters, USA), a column heater (Waters), a carbohydrate separation column (Waters Sugar-Pak column (6.5 x 300 mm), Waters) and a refractive index detector (Waters 2414, Waters). Mobile phase consisted of 50 mg/l Na_2Ca-EDTA, flow rate was set to 0.5 ml/min and column temperature was set to 90°C. The 50 µl injection loop was filled with the sample using a 100 µl LC glass syringe. The analysis was performed in duplicate. The amount of SDFS was calculated using equation 3. (Megazyme 2018a), where Rf is response factor, W_{IS} is weight (mg) of internal standard (D-sorbitol) in 1 ml, PA_{SDFS} is peak area of SDFS, PA_{IS} is peak area of internal standard and M is weight of the sample. Response factor is determined as (PA_{IS}) / ($PA_{Glucose}$) x ($Wt_{Glucose}$) / (Wt_{IS}), where $PA_{Glucose}$ is peak area of D-glucose and $Wt_{Glucose}$ is mass of D-glucose in standard solution.

$$SDFS\left(\frac{mg}{100g}\right) = R_f \times (Wt_{IS}) \frac{(PA_{SDF})}{(PA_{IS})} \times \frac{100}{M} \quad (3)$$

Performance of the method was followed with a VTT rye bran standard sample. Total dietary fibre (TDF) content was calculated as a sum of insoluble and soluble dietary fibre with equation 4 (ANKOM 2016), where R_1 and R_2 are weights of the residues for the duplicate samples (g), P is protein residue (g), A is ash residue (g), B is blank value, and M_1 and M_2 are the original sample weights (g). Blank values for IDF and SDF bags were provided by ANKOM (2016) and were -0.0052 and -0.0045, respectively. The error of the IDF and SDFP fractions was calculated as the average deviation of the recovered residue values divided by the average of the residue values and then multiplied with the final dietary fibre fraction content.

$$TDF(\%) = \left[\left(\frac{\left[\frac{(R_1 + R_2)}{2}\right] - P - A - B}{\frac{(M_1 + M_2)}{2}}\right) \times 100\right] + SDFS\ (\%) \quad (4)$$

2.1.3.2 Starch analysis by enzymatic methods

In this Master's thesis, starch properties were analysed with enzymatic assays combined with spectroscopy suitable for whole grain flours. The basic principle of these three methods (damaged starch content, total starch content, amylose and amylopectin content) is that D-glucose released by hydrolysis of starch reacts with glucose oxidase-peroxidase reagent containing 4-aminoantipyrine and p-hydroxybenzoic acid (GOPOD). D-glucose is oxidised to D-gluconate with release of hydroperoxide that forms a colourful quinoeimine dye in reaction with p-hydrobenzoic acid and 4-aminoantipyrine (equations 5 and 6). Formation of quinoeimine dye is measured with spectrophotometer set at 510 nm. The following equipment were used in all enzyme assay methods; spectrometer (UV-1800, Shimadzu, Japan) water bath with heating circulator (Optima TC120, Grant Instruments, England), analytical balance (Sartorius research R300S, Sartorius, Germany), vortex mixer (Vortex Genie 2, Scientific industries, USA) and centrifuge (5810R, Eppendorf AG, Germany). The performance of the methods was followed with reference materials provided in the enzyme kits.

$$D\text{-}Glucose + O_2 + H_2O \xrightarrow{(glucose\ oxidase)} D\text{-}Gluconate + H_2O_2 \quad (5)$$

$$2H_2O_2 + p\text{-}hydrobenzoic\ acid + 4\text{-}aminoantipyrine \xrightarrow{(peroxidase)} quinoneimine\ dye + 4H_2O \quad (6)$$

Total starch analysis

Total starch content of the fine fractions obtained in air-classification were analysed according to AOAC Method 996.11 with MEGAZYME© Total starch assay procedure (product K-TSTA-100A 04/19). First, 100 mg of sample was weighed into four sample tubes from which one represented the sample blank and then 10 ml of 100 mM sodium acetate buffer (pH 5.0) was added in tubes and tubes were mixed for five seconds with vortex mixer. Starch was hydrolysed to maltodextrins by adding 0.1 ml of undiluted thermostable α-amylase (2500 Ceralpha units/ml reagent at pH 5) into three sample tubes whereas 0.1 ml of 100 mM sodium acetate buffer was added into sample blank. Tubes were mixed for three seconds with a vortex mixer, transferred into a boiling water bath and incubated for 15 min. All tube contents were mixed vigorously after 2-, 5- and 10-minute time points and after removal from the water bath at 15 min. Tubes were transferred into a water bath set at 50°C and allowed to equilibrate for 5 min.

Maltodextrins were further hydrolysed to D-glucose by adding 0.1 ml of undiluted amyloglucosidase (3300 U/ml) into the three sample tubes and 0.1 ml of 100 mM sodium

acetate buffer was added into sample blank. All tube contents were mixed for 3 seconds and incubated at 50°C for 30 minutes. Tubes were moved from the water bath and allowed to cool to ambient temperature over 10 min after which 2 ml of each sample was transferred to 2 ml microfuge tubes and centrifuged at 16 200 g for 5 min at 22°C. After centrifugation, 1 ml of the supernatants were mixed with 10 ml of 100 mM sodium acetate buffer and mixed well. 0.1 ml of these dilutions were transferred to glass test tubes and incubated with 3 ml of GOPOD reagent at 50 °C for 20 min in order to obtain the quinoneimine dye. Absorbances were measured against reagent blank (0.1 ml 100 mM sodium acetate buffer, 3 ml GOPOD reagent) with spectrophotometer set at 510 nm. Quadruplicates of glucose controls containing 0.1 ml D-glucose standard solution (1.0 mg/ml) and 3 ml of GOPOD reagent were incubated concurrently with the samples. Performance of each determination was followed with a standard maize starch sample provided in the analysis kit. Starch content of the samples was calculated according to equation 7 (Megazyme 2019), where ΔA is the mean absorbance of the sample triplicates calculated as the subtraction of the actual sample absorbance and sample blank absorbance, F is the conversion from absorbance to µg of D-glucose, EV is the sample extraction volume (10.2 ml), D is final dilution of the sample (11), W is the sample weight and 0.9 is the factor derived from conversion from micrograms to milligrams and conversion from free glucose to anhydroglucose.

$$Starch\ (\%) = \ \Delta A\ x\ F\ x\ EV\ x\ \frac{D}{W}\ x\ 0.9 \qquad (7)$$

Amylose and amylopectin analysis

Amylose content was determined as triplicates with a MEGAZYME© Amylose/Amylopectin assay procedure (product K-AMYL 06/18). First, 20-25 mg of sample was weighed with an analytical balance in triplicates into glass test tubes. Then, 1 ml of dimethyl sulphoxide (DMSO) was added into each tube, tubes were mixed gently and heated in a boiling water bath until the samples were completely dispersed (approximately 1 min). The samples were further heated in a boiling water bath for 15 min with occasional high-speed stirring on a vortex mixer. Tubes were removed from water bath and allowed to cool to ambient temperature. Lipids were removed by adding first 3 ml of 95% (v/v) ethanol into the tubes with continuous mixing and then adding 3 ml more of 95% (v/v) ethanol and inverting the tubes to ensure the formation of a starch precipitate. Tubes were allowed to stand for overnight to ensure the complete precipitation of starch.

On the following morning, sample tubes were centrifuged at 2000 g for 5 min at 22°C and lipid containing ethanol was drained from tubes. Remaining starch pellet was mixed with 2

ml of DMSO and heated in boiling water bath for 15 min with occasional mixing. On the removal from water bath, 4 ml of sodium acetate buffer was added to the tubes and the tubes were mixed thoroughly. Tube contents were removed by repeated washing with sodium acetate buffer into 25 ml volumetric flasks. Flasks were filled to mark with sodium acetate buffer. As an exception to method instructions, samples were centrifuged at 1850 g for 10 min at 22°C, instead of filtering, to sediment the other components than starch. One ml of the supernatant from this centrifugation was transferred to 2 ml microfuge tubes and mixed carefully with 0.5 ml of concanavalin A (Con A) solution to precipitate the amylopectin. Microfuge tubes were incubated for 60 min at ambient temperature and centrifuged at 14000 g for 10 min at 22°C. One ml aliquots of the supernatants now containing the amylose were transferred into 10 ml glass test tubes and mixed with 3 ml of 100 mM sodium acetate buffer (pH 4.5) and heated in boiling water bath to denature the Con A. Tubes were transferred into a water bath set at 40°C and allowed to equilibrate for 5 min. Remaining amylose was hydrolysed into D-glucose by adding 0.1 ml of both α-amylase (25 Ceralpha units/ml) and amyloglucosidase (25 U/ml) mixture into each test tube and incubating the tubes for 30 min at 40°C. After incubation, the tubes were centrifuged at 2000 g for 5 min and 1 ml aliquots were incubated with 4 ml of GOPOD-reagent for 20 min at 40°C.

To obtain the absorbance representing the total starch content of the solution, 0.5 ml of the aliquot obtained from the 1850 g centrifugation was mixed with 4 ml of 100 mM sodium acetate buffer (pH 4.5) and incubated with 0.1 ml of α-amylose (25 Ceralpha units/ml) and amyloglucosidase (165 U/ml) mixture. One ml of the solution was transferred to glass test tubes and incubated with 4 ml of GOPOD reagent 20 min at 40°C. This incubation was performed concurrently with the tubes containing only amylose. Absorbances were measured at 510 nm against reagent blank containing 1 ml of sodium acetate buffer and 4 ml of GOPOD-reagent. D-glucose controls were incubated in duplicates with GOPOD-reagent to ensure the correct formation of quinoneimine dye. A maize starch control sample was analysed with each determination to follow the performance of the method. Amylose content was calculated based on the absorbance values obtained for Con A supernatant and acetate salt solution aliquot with equation 8 (Megazyme 2018b) with dilution factor of 66.8.

$$Amylose\ (\%, w/w) = \frac{Absorbance\ (Con\ A\ supernatant)}{Absorbance\ (Total\ starch\ aliquot)} \times dilution\ factor \qquad (8)$$

Damaged starch analysis

Damaged starch content was analysed according to the AACC 76-31.01 method described originally by Gibson et al. (1992) and evaluated by Gibson et al. (1993) using a MEGAZYME© Damaged starch assay kit. First 100 ± 10 mg of each sample was weighed into glass test tubes in quadruplicates, from which three were treated with enzymes and the fourth presented the sample blank. Damaged starch granules were first hydrated and hydrolysed to maltosaccharides and dextrins by adding 1 ml of fungal α-amylase (50 U/ml) and incubating at 40°C exactly for 10 min. One ml of 100 mM sodium acetate buffer was added to sample blank instead of α-amylase. Incubation was followed with a stopwatch. This reaction was terminated by addition of 8 ml of 0.2% (v/v) sulphuric acid. Tubes were centrifuged at 1000 g for 5 min at 22°C and 0.1 ml of supernatants from the centrifugation were transferred into the bottom of new glass test tubes.

Complete degradation of dextrins to D-glucose in the aliquot was carried out by adding 0.1 ml of amyloglucosidase (20 U/ml) into each tube followed by incubation at 40°C for 10 min. To sample blanks, 0.1 ml of sodium acetate buffer was added instead of amyloglucosidase. Four ml of GOPOD reagent was added to tubes and tubes were incubated at 40°C for 20 min. Three tubes containing 0.1 ml of glucose standard, 0.1 ml sodium acetate buffer and 4 ml of GOPOD reagent were incubated concurrently with the samples to obtain the absorbance values for the D-glucose. Sample absorbance was measured with spectrophotometer at 510 nm against the reagent blank containing 0.2 ml of sodium acetate buffer and 4 ml of GOPOD reagent. Damaged starch content was calculated according to equation 9 (Megazyme 2018c), where ΔE is absorbance of the sample, F (150 µg of glucose/ absorbance of 150 µg of glucose) is conversion from absorbance to micrograms, W is the weight of the sample and 8.1 is the factor derived from dilutions, conversion from micrograms to milligrams and conversion from free glucose to anhydroglucose. A reference wheat sample was analysed with each set of determination to follow the performance of the method.

$$Damaged\ starch\ (\%,) = \Delta E\ x\ \frac{F}{W}\ x\ 8.1 \qquad (9)$$

2.1.3.3 RVA analysis

Pasting properties of the oat samples were analysed using Rapid Visco Analyser (RVA Super 4 by Newport Scientific, Warriewood, Australia). RVA was chosen as a suitable method in this study because it has good repeatability and reliability, and the analysis time is shorter

compared to the other instruments used for measuring pasting properties. RVA analysis followed the Standard Newport Scientific method 1 (STD1) as described the RVA handbook by Crosbie and Ross (2007). The STD1 method was chosen since it is commonly used method in RVA analysis and therefore, the results are more comparable with the results from other studies as well. In the beginning of the analysis, sample and RO water were weighed and mixed in sample tins. The required sample amount was calculated based on the sample moisture content so that the sample and water mixture contained 25 g of water and 3.5 g of dry sample yielding dry matter content of 12.3%. The analysis was initiated by mixing samples for 10 seconds at paddle speed of 960 rpm followed by 50 s holding period with paddle speed of 150 rpm. The paddle speed was held at 150 rpm for the rest of the measurement. Mixing step was followed by heating step from 50 to 95°C during 282 seconds. Temperature was held at 95°C for 150 seconds and then the sample was cooled back to 50°C during 228 seconds. All samples were analysed in triplicate. In this thesis, the apparent viscosities measured with RVA are referred to as viscosity.

2.1.3.4 Statistical analysis

All results, average deviations, coefficients of variations and Spearman's correlations were calculated using Excel spreadsheet software (Excel 2016, Microsoft, Redmond, US). Differences between damaged starch contents and pasting properties in G samples, NFL samples and air classified fine fractions were analysed with one-way analysis of variance (ANOVA) with Tukey's honestly significant difference (HSD) ($p<0.05$) posthoc test. Independent sample T-tests ($p<0.05$) were performed to NFL and fine fraction amylose results to study the difference between the samples. ANOVA and T-tests were performed with SPSS-software (IBM SPSS Statistics, version 26, IBM, New York, US).

2.2 Results

2.2.1 Dietary fibre content of oat flours

Total dietary fibre content of NFL samples varied between 9.5-13.1% in dry matter basis (Figure 5). IDF content of NFL samples was 3.9-5.3%, SDFP content 3.7-5.7% and SDFS content 1.0-4.1% in dry matter basis. Thus, IDF fraction represented 38-50%, SDFP fraction 30-49% and SDFS fraction 10-32% of the TDF content in the oat samples. Sample NFL003 had both the highest IDF content (5.0% dm) as well as the highest SDFS content (4.1% dm) compared to other NFL samples. SDFS content in most of the samples varied between 1-2% in dry matter basis.

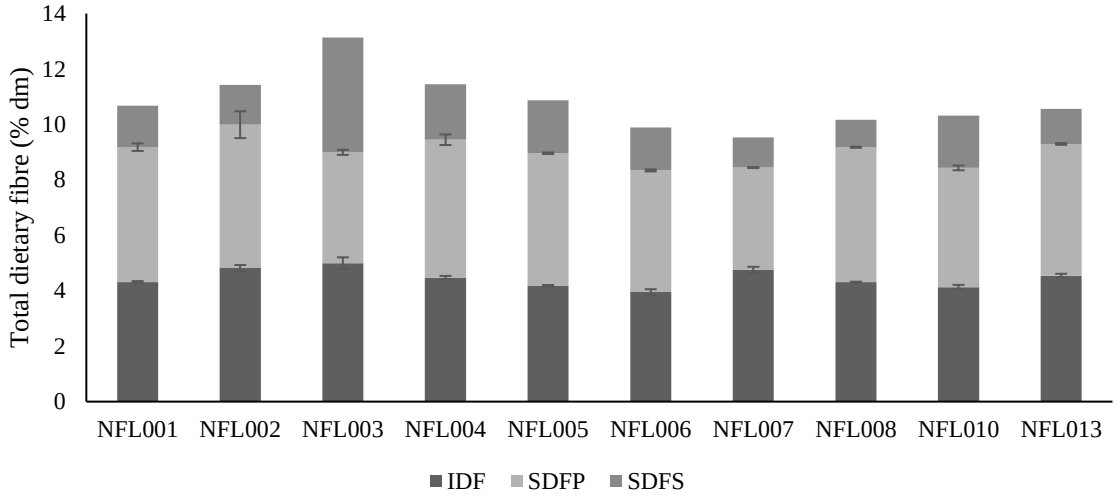

Figure 4. Total dietary fibre (TDF) content of oat flours (NFL) expressed as insoluble dietary fibre (IDF), soluble dietary fibre that precipitates in ethanol (SDFP) and soluble dietary fibre that stays soluble in 78% ethanol (SDFS) contents. Numbers 001-013 represent the 10 oat samples. Dietary fibre was analysed duplicate, and the error was estimated based on the deviations calculated from the recovered dietary fibre residues obtained in the analysis, after which the ash content of one of the duplicate samples and protein content of the other was analysed. SDFS fractions were analysed without duplicates.

2.2.2 Starch properties of non-heat-treated oat groats and oat flour

Damaged starch content varied between 1.2-1.6% (dm) of total starch in G samples (Table 2). G004 sample had significantly ($p<0.05$) lower and G003 had significantly higher amount of damaged starch compared to other G samples. Content of damaged starch was significantly ($p<0.05$) (data not shown) higher in NFL samples than in G samples and varied between 5.6-10.5% (dm). Sample NFL002 had significantly ($p<0.05$) lower and NFL008 significantly ($p<0.05$) higher damaged starch content than other NFL samples. The difference between damaged starch content of G and NFL samples was 3- to 7-fold depending on the sample. Amylose content of NFL samples varied between 19.2-25.7% of starch weight (Table 2). Sample NFL002 had significantly ($p<0.05$) lower amylose content than the other NFL samples. Content of SDFP showed a negative correlation ($p<0.05$) with amylose content in NFL flours (Table 5).

Table 2. Damaged starch content of non-heat-treated oat groats (G) and damaged starch and amylose contents of mill processed (drying, dehulling, kilning, flaking and milling) oat flours (NFL). Values are expressed as means (n=3) ± standard deviation.

Sample	G	NFL	
	Damaged starch % dm / starch	Damaged starch % dm / starch	Amylose % /starch
001	1.47 ± 0.03 e	6.82 ± 0.09 c	24.0 ± 0.2 bc
002	1.47 ± 0.01 e	5.64 ± 0.01 a	19.2 ± 0.9 a
003	1.55 ± 0.01 f	9.70 ± 0.11 g	25.7 ± 0.2 c
004	1.22 ± 0.01 a	6.65 ± 0.14 c	22.1 ± 0.8 b
005	1.38 ± 0.02 cd	7.81 ± 0.14 de	24.6 ± 0.8 c
006	1.47 ± 0.02 e	8.09 ± 0.09 e	24.0 ± 0.1 bc
007	1.34 ± 0.02 bc	7.61 ± 0.16 d	25.4 ± 1.1 c
008	1.33 ± 0.02 bc	10.5 ± 0.04 h	25.7 ± 0.5 c
010	1.40 ± 0.02 d	8.75 ± 0.23 f	24.1 ± 0.7 bc
013	1.29 ± 0.03 b	6.01 ± 0.03 b	22.0 ± 1.6 b

Different letters within each column indicate statistically significant difference ($p<0.05$) between samples based on Tukey's HSD test.

2.2.3 Air classification and starch properties of the fractions

Fine (NFL-F) and coarse (NFL-C) fractions obtained by air classification from industrially milled oat flours had mass yields of 71.1-79.8% and 16.8-25.0%, respectively (Table 3). Total starch content of the fine fractions 71.1-84.0% (dm) was higher than the content in the original NFL samples (58.4-71.9% dm)(Table 1). The total starch content of NFL samples showed positive correlation ($p<0.05$) with the mass yields of the NFL-F samples (Table 5). Starch separation efficiency (SSE) from the raw material to the fine fraction was 87.8-94.3%. Damaged starch constituted 4.8-8.9% of starch weight in the fine fractions and was significantly lower ($p<0.05$) when compared to NFL samples (data not shown). Amylose contents of the NFL-F samples were 22.6-27.1% (dm) and samples NFL001F and NFL002F had significantly higher ($p<0.05$) amylose content than the original NFL samples. This was not observed between NFL003-013 and NFL003F-013F. A statistically significant positive correlation ($p<0.05$) was observed between starch damage and amylose contents in air-classified fine fractions (Table 6).

Table 3. Mass yields, total starch, damaged starch and amylose contents and starch separation efficiencies of fine fractions (NFL-F) and mass yields of coarse fractions (NFL-C) produced by air classification (British Rema Manufacturing company Ltd., UK) with air classifier wheel speed of 2500 rpm and airflow of 220 m^3/h. Mass yields are expressed as average values (n=2) ± average deviation and starch, amylose and damaged starch contents are shown as average values (n=3) ± standard deviation.

Sample	Fine fraction NFL-F					Coarse fraction NFL-C	Lost
	Mass yield (%, dm)	Total starch (%, dm)	Damaged starch (%/starch)	Amylose (%/starch, w/w)	Starch separation efficiency (%, dm)	Mass yield (%, dm)	Mass yield (%, dm)
NFL001	71.7 ± 0.2	75.5 ± 2.3	4.8 ± 0.1 a	25.7 ± 0.6	87.8 ± 0.3	25.0 ± 0.1	3.3
NFL002	73.4 ± 0.3	72.1 ± 0.5	5.1 ± 0.1 a	23.3 ± 0.5	88.6 ± 0.5	24.0 ± 0.1	2.6
NFL003	76.6 ± 0.1	77.9 ± 0.5	8.6 ± 0.4 e	26.7 ± 0.7	90.6 ± 0.2	19.9 ± 0.0	3.5
NFL004	78.5 ± 0.9	77.9 ± 1.0	6.4 ± 0.1 b	22.6 ± 0.4	90.3 ± 1.0	18.2 ± 0.3	3.3
NFL005	75.2 ± 0.6	80.5 ± 1.1	6.9 ± 0.1 bc	25.3 ± 0.3	91.9 ± 0.7	20.9 ± 0.0	3.9
NFL006	79.1 ± 0.5	84.0 ± 0.3	7.4 ± 0.1 c	24.6 ± 0.6	92.4 ± 0.6	17.2 ± 0.4	3.7
NFL007	72.7 ± 0.6	82.9 ± 1.9	6.5 ± 0.2 b	25.2 ± 1.7	86.3 ± 0.7	23.0 ± 0.3	4.3
NFL008	79.8 ± 0.1	83.3 ± 1.4	8.9 ± 0.2 e	27.1 ± 1.0	94.3 ± 0.1	16.8 ± 0.9	3.4
NFL010	77.1 ± 1.5	80.2 ± 0.2	7.9 ± 0.1 d	24.5 ± 0.1	89.7 ± 1.8	19.4 ± 1.2	3.5
NFL013	71.9 ± 1.6	73.1 ± 2.7	5.3 ± 0.1 a	23.9 ± 0.8	89.9 ± 2.0	24.7 ± 0.7	3.4

Different letters within each column indicate statistically significant difference ($p < 0.05$) between samples based on Tukey's HSD test.

2.2.4 Pasting properties

In general, the pasting properties of the 10 oat raw materials changed due to processing. The milling process increased the peak viscosity (PV), through viscosity (TV), setback viscosity (SBV), final viscosity (FV) values and time to peak viscosity values (TTPV) and did not affect the breakdown viscosity (BDV) or pasting temperature (PT) values (Table 4). The G samples exhibited significantly ($p<0.05$) lower PV for all samples compared to NFL samples. Examples of changes in pasting behaviour are shown in Figure 6. TTPV values of G samples were significantly ($p<0.05$) lower compared to NFL samples (data not shown). TTPV values of G samples varied between 5.5 and 6.2 min and in NFL samples between 5.7 and 6.8 minutes. Pasting properties of fine fractions obtained by air classification did not differ from NFL samples except in regard to BDV and SBV values. NFL-F samples had either higher PV values or similar PV values as NFL samples. The fine fraction exhibited significantly ($p<0.05$) higher BDV values when compared to G and NFL samples.

Table 4. Effect of milling process and air classification on pasting properties (peak viscosity, PV; through viscosity, TV; breakdown viscosity, BD; final viscosity, FV; setback viscosity, SB; pasting temperature; PT) of oat. Values are presented as average values (n=30) ± standard deviation.

	PV (mPa*s)	TV (mPa*s)	BDV (mPa*s)	FV (mPa*s)	SBV (mPa*s)	PT (°C)
G	3746 ± 471 a	1816 ± 359 a	1930 ± 199 a	4273 ± 656 a	2456 ± 471 a	84.1 ± 1. 9a
NFL	4614 ± 439 b	2690 ± 285 b	1923 ± 339 a	5943 ± 1065 c	3253 ± 1016 b	82.4 ± 3.7 a
NFL-F	4862 ± 467 b	2576 ± 310 b	2286 ± 308 b	5352 ± 841 b	2776 ± 821 ab	83.4 ± 3.7 a

Different letters within each column indicate statistically significant difference ($p < 0.05$) between treatments (G, NFL, fine fractions) based on Tukey's HSD test.

Pasting profile values for all the samples are presented in Appendix 1. G samples exhibited the lowest ($p<0.05$) final viscosity values in all other samples than in sample G001 (Figure 6A.), where fine fraction had the lowest final viscosity. Air classified fine fraction NFL001F had lower TV and TTPV values than G001 and NFL001, whereas for all other samples the TV and TTPV values of G samples had the lowest values. Samples NFL002, NFL004 and NFL006 had higher TV values than corresponding NFL-F samples whereas no significant difference in the rest of the samples was observed. In all samples other than G001, the G samples had the lowest TTPV values. The NFL samples exhibited the highest setback values compared to G and fine fraction samples in all other samples except in sample 003. In sample 003 the SB values of G, NFL and fine fraction samples were not significantly different. Sudden decreases were observed in the viscosity values of samples NFL002, NFL002F, NFL004 and NFL004F during the second holding stage (Figure 6B, D).

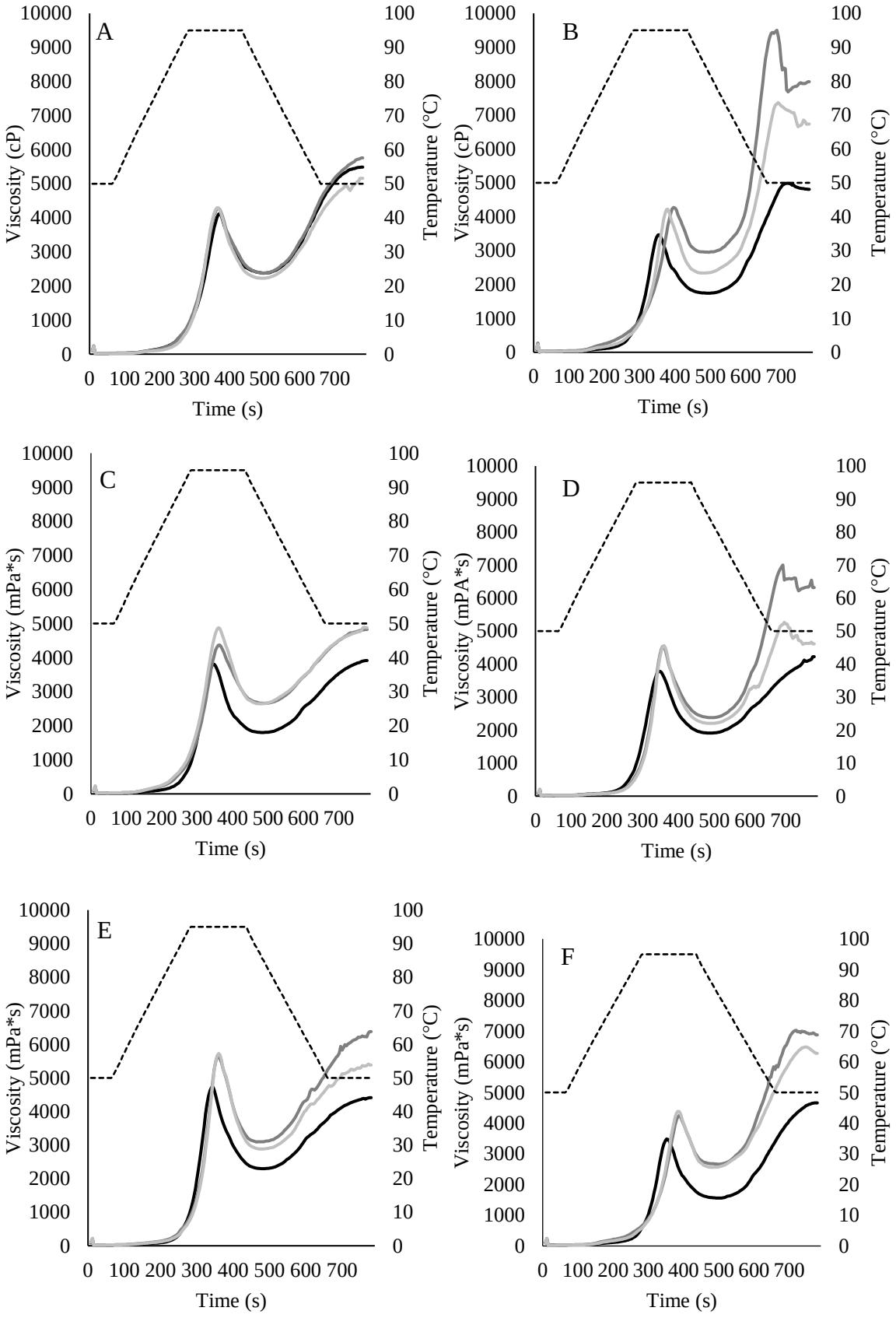

Figure 5. Examples of effect of processing on oat pasting properties in samples 001 (A), 002 (B), 003 (C) 004 (D), 006 (E) and 013 (F). Sample types were non-heat-treated oat groats (G, black line), oat flour (NFL, dark grey line) and fine fraction produced with air classification from NFL flours with air classifier wheel speed of 2500 rpm and airflow of 220 m^3/h (NFL-F, light grey line).

Starch components and dietary fibre fractions showed significant correlations with oat flour pasting parameters (Table 5). Total starch content of NFL and NFL-F samples correlated positively ($p<0.05$) with PV and BDV values. A significant negative correlation ($p<0.05$) in all samples, G, NFL and NFL-F, was observed between total starch content and SBV values. In addition, the total starch content of NFL samples correlated negatively ($p<0.05$) with TTPV values. Amylose content of NFL samples was observed to have significant ($p<0.01$) negative correlation with FV and SBV values. This was not observed in NFL-F samples (Table 6.). Amylose content showed significant ($p<0.05$) negative correlation with PT in fine fractions. SDFP fraction of TDF showed strong positive correlation ($p<0.01$) with FV and SBV, positive correlation ($p<0.05$) with TTPV values and negative correlation ($p<0.05$) with amylose content in NFL flours. Damaged starch showed negative correlation ($p<0.05$) with setback values in both NFL samples and fine fractions. Additionally, damaged starch correlated positively ($p<0.05$) with PV values of fine fractions. There were no significant correlations between G sample pasting parameters and damaged starch content (data not shown).

Table 5. Correlations between oat flour (NFL) carbohydrate components, air classification parameters and pasting properties calculated as Spearman correlation coefficients (n=10). Abbreviations: Peak viscosity (PV), through viscosity (TV), breakdown viscosity (BDV), final viscosity (FV), setback viscosity (SBV), time to reach peak viscosity (TTPV), peak temperature (PT), insoluble dietary fibre (IDF), water soluble dietary fibre that precipitates in 78% ethanol (SDFP), water soluble dietary fibre that stays soluble in 78% ethanol (SDFS), mass yield of air-classified fine fraction (MY), starch separation efficiency (SSE).

	PV	TV	BDV	FV	SBV	TTPV	PT	Total starch	Amylose	Starch damage	IDF	SDFP	SDFS	TDF	MY
PV	1.00														
TV	0.62	1.00													
BDV	0.75*	-0.05	1.00												
FV	-0.08	0.31	-.0.37	1.00											
SBV	-0.26	0.05	-0.37	0.96**	1.00										
TTPV	-0.45	0.25	-0.79*	0.79*	0.76*	1.00									
PT	0.22	-0.16	0.42	-0.29	-0.26	-0.47	1.00								
Total starch	0.77*	0.29	0.74*	-0.57	-0.68*	-0.80*	0.32	1.00							
Amylose	0.33	0.15	0.29	-0.83**	-0.92**	-0.63	0.38	0.64	1.00						
Starch damage	0.41	0.42	0.17	-0.66*	-0.81*	-0.53	0.04	0.68*	0.82**	1.00					
IDF	-0.57	-0.33	-0.45	-0.18	-0.10	0.21	-0.33	-0.32	0.02	-0.03	1.00				
SDFP	-0.26	0.17	-0.47	0.86**	0.86**	0.68*	-0.36	-0.55	-0.77*	-0.50	-0.24	1.00			
SDFS	-0.22	-0.18	-0.13	-0.33	-0.30	-0.24	-0.18	0.00	0.25	0.34	0.48	-0.31	1.00		
TDF	-0.49	-0.16	-0.49	0.13	0.18	0.22	-0.45	-0.39	-0.20	0.01	0.58	0.20	0.83	1.00	
MY fine fraction	0.67*	0.47	0.46	-0.17	-0.31	-0.53	0.15	0.75*	0.30	0.65*	-0.35	-0.07	0.20	0.03	1.00
SSE	0.53	0.77*	0.02	0.14	-0.07	-0.06	0.17	0.38	0.25	0.57	-0.42	0.17	0.08	0.04	0.77*

Significance level: *, $p < 0.05$; **, $p < 0.01$

Table 6. Correlations between starch, damaged starch and amylose content and pasting properties of fine fractions produced with air classification from oat flours with air classifier wheel speed of 2500 rpm and airflow of 220 m3/h calculated as Spearman correlation coefficients (n=10). Abbreviations: Peak viscosity (PV), through viscosity (TV), breakdown viscosity (BDV), final viscosity (FV), setback viscosity (SBV), time to reach peak viscosity (TTPV) and peak temperature (PT) and mass yield of air-classified fine fraction (MY).

	PV	TV	BDV	FV	SBV	TTPV	PT	Starch content	Amylose	Starch damage
PV	1.00									
TV	0.76*	1.00								
BDV	0.76*	0.14	1.00							
FV	-0.27	0.25	-0.66*	1.00						
SBV	-0.57	-0.13	-0.73*	0.93**	1.00					
TTPV	-0.37	0.21	-0.77*	0.90**	0.84**	1.00				
PT	-0.34	-0.34	-0.17	0.37	0.50	0.33	1.00			
Starch content	0.93**	0.63	0.77*	-0.48	-0.73*	-0.50	-0.30	1.00		
Amylose	0.42	0.62	0.02	-0.14	-0.38	-0.08	-0.75*	0.45	1.00	
Starch damage	0.74*	0.64	0.48	-0.43	-0.68*	-0.47	-0.70*	0.71*	0.57	1.00
MY fine fraction	0.65*	0.48	0.51	-0.32	-0.51	-0.51	-0.26	0.62	0.14	0.79*

Significance level: *, $p < 0.05$; **, $p < 0.01$

2.3 DISCUSSION

In this thesis, the carbohydrate properties of non-heat-treated oat groats, oat flour and air-classified fine fraction produced from oat flour were analysed. The dietary fibre, starch and amylose contents of oat flour were determined to understand better the carbohydrate content and composition of oat flour. Damaged starch content of the three sample types was determined to investigate the possible impact of oat milling and air classification on oat starch quality. The air classification of the industrially processed oat flours was performed in order to evaluate differences between different samples in mass yields of the fine and coarse fractions and starch separation efficiency to the fine fraction. Furthermore, the physiochemical properties were measured with Rapid Visco Analyser (RVA) to understand the pasting properties of oat samples and possible process-induced changes in the those properties.

2.3.1 Carbohydrate properties of oat flour samples

As hypothesised, variation between the shares of dietary fibre fractions in the NFL samples was observed. One interesting sample was NFL003 that had considerably higher SDFS content (4.1% dm) than the other NFL samples (1.0-2.0% dm). The total dietary fibre contents (9.5-13% dm) and the variation between different oat samples found in this study are in agreement with previously reported values. Manthey et al. (1999) reported TDF values of 10.2-12.1% (dm) in six US oat varieties. In their study, SDF presented 38-42% and IDF 58-62% of TDF, which differ from what was seen in the current research where lower share of IDF was observed. The difference may be explained by the change in the definition of the dietary fibre as well as by the different analysis methods. The definition of the dietary fibre has changed during past decades as new dietary fibre components have been found and included in it, thus results from previous studies can be based on different definitions (McCleary et al. 2012; McCleary et al. 2013). The AOAC method 2011.25 used in the current study includes the oligosaccharides into SDF fraction as well, whereas Manthey et al. (1999) analysed the TDF content without the oligosaccharides. Also higher TDF contents have been reported in the literature for commercial oat flours. Hüttner et al. (2010) reported TDF content of 17.8-19.2% (dm) in commercial oat flours from Finland, Ireland and Sweden analysed with AOAC Method 991.43.

Similarly as observed in the current study, TDF values of 11.0-12.6% (dm) in whole oat flakes and 9.2% (dm) in industrial oat flour samples have been reported by Rainakari et al. (2016). However, Rainakari et al. (2016) reported higher IDF contents (6.7-8.1% dm),

similar SDFP contents (3.7-5.6% dm) and at least two times smaller SDFS contents (0.2-0.5%) in the oat flakes than what was found in the current study (3.9-5.3%, 3.7-5.7%, and 1.0-4.1%). As the analysis method is the same, the variation may result from the difference between the semi-automated method used in the current study and the manual method used by Rainakari et al. (2016). One possible explanation to the lower IDF contents and higher SDFS contents obtained in this study could be that the filtering and mixing steps used in current study are more efficient compared to the manual method and more dietary fibre components are transferred to the SDFP and SDFS fractions. In addition to differing analytical methods, the differing proportions of the bran particles in oat flour samples can explain the different dietary fibre contents of oat flours reported in the literature.

There is a large variation in the amylose contents of oat starch previously reported in the literature, which is explained by the different analytical methods used for determining the amylose content in cereal grains (Hoover et al. 2003; G. Hu et al. 2010; Hüttner et al. 2010; Nguyen et al. 2019). Regarding amylose content, the ten oat flour samples (NFL) are in the same range with the values previously reported in the literature. Hu et al. (2010) compared the conventional colorimetric iodine method to amylose content method by Megazyme. The iodine method is based on measuring the formation of blue amylose iodine complex with spectrophotometer at various wavelengths (Peris-Tortajada 2004). Hu et al. (2010) observed that the iodine-based method yielded lower values in regular amylose content samples (amylose content 20% or more). Hüttner et al. (2010) determined the amylose content of three commercial oat flours with amylose content method by Megazyme and reported values between 28.8-31.6% (dm). These results are higher than obtained in the current study. Higher amylose contents can be explained by different sample origins as the amylose content varies between different oat genotypes (Autio and Eliasson 2009; Zhu 2017). Nguyen et al. (2019) analysed amylose content of heat-treated oat groats with size exclusion (SEC) chromatography and reported contents as high as 37.5%. They remarked that SEC has been observed in other studies to have systematic differences with iodometric methods. In the literature, oat starch amylose content has often been determined from purified oat starch samples as other components such as lipids and proteins can interfere with the analysis (Hoover et al. 2003; Peris-Tortajada 2004; Stevenson et al. 2007). In this study, the amylose content determination method by Megazyme was chosen based on its suitability for the whole oat samples containing also other grain components in addition to starch.

Ten NFL samples had very different damaged starch contents as sample NFL008 (10.6%) had almost twofold amount compared to sample NFL002 (5.9%). Starch damage can occur

during milling and it has been reported to affect the physicochemical properties of cereal flours and starches (Boyaci et al. 2004; León et al. 2006; Hüttner et al. 2010; Assatory et al. 2019). Only a little research is available regarding damaged starch content in commercial oat flours. Hüttner et al. (2010) analysed damaged starch contents of 1.6, 6.7 and 9.2% in three commercial oat flours. They reported that the damaged starch content affected the rheological properties and baking quality of the oat flour. The oat flour sample with the highest amount of starch damage had inferior baking quality compared to two other oat flours. According to Hüttner et al. (2010), a moderate amount of damaged starch can be also beneficial for baking quality. Based on these findings damaged starch content may potentially have a negative impact on baking quality of samples NFL003 and NFL008 that had high contents of damaged starch.

It can be concluded that the ten different oat flour samples differed in their dietary fibre content, amylose content and damaged starch content as it was hypothesised. The obtained results were mainly in agreement with the previously reported values in the literature.

2.3.2 Effect of milling process on oat flour starch and pasting properties

As expected, the milling process inflicted damage to oat starch and affected the oat flour pasting properties. Damaged starch contents of the non-heat-treated oat groat samples were sample dependent as they differed significantly from each other. As milling can cause starch damage, it may be estimated that almost all starch damage in G samples originated from the lab-scale milling (Morgan and Williams 1995; Boyaci et al. 2004). Therefore, it can be assumed that the intact oat groats contained almost no damaged starch. The difference between the damaged starch content of oat flours (NFL) and non-heat-treated oat groats (G) was sample dependent, i.e. the ten samples behaved differently during processing. In wheat, the amount of damaged starch depends on the harshness of the grinding as well as on the hardness of the wheat grain as reviewed by Boyaci et al. (2004). There is little information available in the literature regarding the relation between the oat grain properties and the amount of starch damage occurring during oat milling. In a study by Engelson and Fulcher (2002), a relation between oat grain characteristics and overall groat damage during dehulling was observed. They reported that the β-glucan content and protein content of the oat grain influenced the amount of groat breakage occurring during dehulling.

The pasting parameters of NFL flours were similar as observed in the study by Hüttner et al. (2010) as the reported peak viscosity, through viscosity, breakdown viscosity and pasting temperature values in commercial oat flours were similar compared to the results obtained

in the current study. However, the final viscosity and setback viscosity values were lower and time to peak viscosity values were higher in the study by Hüttner et al. (2010). Comparison of pasting properties between different previously published results is challenging as many different pasting test profiles and conditions have been used. For example in the study by Hüttner et al. (2010) the pasting profile steps (heating, first holding, cooling and final holding step) were altogether longer than in the current study, whereas the temperatures were the same. Similarly, pasting profiles with differing temperature conditions, amounts of sample and test times have been used in other studies (Zhou M. et al. 1999; Choi et al. 2012; Ziegler et al. 2018; Nguyen et al. 2019).

Milling process increased the values of all other pasting parameters except the breakdown viscosity and pasting temperature. The effect of heat treatment and milling on oat pasting properties has also been studied by Zhou M. et al. (1999) who observed similar changes as in this study in the pasting properties of three Australian oat cultivars after small scale processing including steaming, kilning and rolling. The processing increased the peak viscosity, time to peak viscosity and final viscosity values and the changes were characteristic of sample, which was observed also in this study. Zhou M. et al. (1999) did not mention any explanation for these changes. The higher PV values may indicate that the milling process increases the water holding capacity and the water hydration capacity of the oat flour. Water hydration capacity of oat flours is enhanced by the protein content, β-glucan content, high damaged starch content and the small particle size of the flour (Hüttner et al. 2010). Higher final viscosity values after milling indicate that the milling process could increase the tendency of the flour to retrograde.

In the current work, the SDFP fraction of dietary fibre showed positive correlations with final viscosity and setback viscosity in NFL samples. This could be related to β-glucan content as most of the soluble dietary fibre in oats is composed of β-glucan. Wang et al. (2016) studied the effect of environment, cultivar and processing on the physicochemical properties of oat β-glucan in five oat cultivars grown in Canada. They reported that processing including kilning, steaming and flaking increased the apparent viscosity of β-glucan in the oat samples. Although Wang et al. (2016) did not study the pasting properties of the oat flours, similar phenomenon could explain the higher viscosities of the NFL samples compared to G samples found in the current study, as the β-glucan content has been linked to pasting parameters in other studies. According to Liu et al. (2010) there might be a notable interaction between β-glucan and starch that affects the pasting properties of oat flours. They postulated that peak viscosity and through viscosity are more starch-related

whereas the final viscosity can be linked to β-glucan content. As the TDF contents of the G samples were not analysed in the current study, the effect of processing on oat dietary fibre content cannot be addressed.

As the total starch content of the G and NFL was similar for all the studied samples it can be expected that the starch content does not explain the occurred changes in the pasting characteristics inflicted by the milling process. Total starch content showed significant positive correlation with PV in NFL and NFL-F samples, but not in G samples. This indicates that the changes in the pasting properties could be explained by the changes occurred in starch structure rather than in the content during mill process. As the peak viscosity can be linked with water absorption capacity of the sample as reviewed by , the increase in the peak viscosity values could be also partly explained by the increased damaged starch content. Damaged starch content has been related to higher water hydration capacity in oat flours (Hüttner et al. 2010). However, no clear correlations between peak viscosity and damaged starch content were observed in G or NFL samples. Starch pasting properties are also affected by the amylose content of the starch (Balet et al. 2019). Nguyen et al. (2019) observed that heat-moisture treatment produced more shorter amylopectin chains and longer amylose chains and increased the amylose content in oat flours. As amylose content of the G samples was not determined in the current study, the effect of milling process on amylose content cannot be discussed. In the current study the amylose content showed negative correlation with final and setback viscosities. Similar correlation between amylose content and setback viscosity was observed in heat-treated oats in the study by Nguyen et al. (2019) as well.

According to Nguyen et al. (2019), the heat treatment increased the pasting temperature and decreased the breakdown, final and setback viscosity of the oat flours. In current study, the milling process did not affect the pasting temperature or the breakdown viscosity and final and setback viscosities increased. The difference between the results in Nguyen et al. (2019) study and current study can be explained with different processing methods. In the study by Nguyen et al. (2019) the heat treatment included wet steaming at 100°C for 40 minutes, dry heating at 125°C for 10 minutes and drying at 112°C for 30 min and milling was performed with cryogenic-milling process to avoid molecular degradation of starch granules. This highlights the importance of understanding the effect of commercial milling processes as different processing conditions seem to yield very different results.

Based on the results obtained in the current study, the milling process can cause a significant amount of starch damage and affect the pasting properties of oats. Furthermore, the oat

samples produced from a single oat variety behaved differently during processing and therefore the effect of milling process on oat properties seems to be raw material dependent. Milling process caused significant changes in all of the pasting parameters except in pasting temperature, but no distinct explanation for these changes can be drawn based on the current data.

2.3.3 Effect of dry fractionation on oat flour properties

The aim of the air classification procedure applied in the current work was to separate the bran particles from the endosperm flour (Sibakov et al. 2011; Sibakov et al. 2012). Dietary fibre-rich bran particles were targeted to be separated into the coarse fraction and starch-rich endosperm particles to enrich into the fine fraction. Mass yields of the fine fractions (NFL-F) from air classification varied between 70 and 80%, which was in line with the targeted yields. Total starch content of NFL showed positive correlation with the mass yield of the NFL-F samples indicating that the mass yield of the starch-rich endosperm fraction may be estimated based on the original starch content of the oat flour. As expected, starch was enriched into the fine fractions. The obtained starch contents of the fine fractions in the current study (71.1-84.0% dm) were higher than in the study by Sibakov et al. (2011) where they obtained the total starch content of 69.8% in fine fraction. Oat flour used in the study by Sibakov et al. (2011) was non-heat-treated and they had removed the lipids from the oat flour in order to enhance the separation of β-glucan, which may explain the difference compared to the current results.

The starch quality and pasting properties of the fine fractions (NFL-F) differed from the original oat flour (NFL) and significant correlations between mass yields and carbohydrate characteristics of NFL-F samples were observed. The NFL-F samples had higher the breakdown viscosity and lower final viscosity than NFL samples. The observed decrease in final viscosity due to air classification could originate from the lower β-glucan content in the fine fraction as beta-glucan is known to enrich in the coarse bran fraction. Damaged starch content was significantly lower in NFL-F samples than in the original NFL samples. Wu and Stringfellow (1992) found in their study that damaged starch content of air classified wheat flour fractions decreased when particle size increased. Their result indicates that flour material with smaller particle size would have higher damaged starch content, which is the opposite of the result observed in the current study. The two possible explanations could be that starch damage occurs differently in wheat starch granules than in the oat starch granules or that part of the smaller particles with higher amount of damaged starch ended up in the lost fraction in the current study. Damaged starch content of NFL-F samples showed similar

correlations with the pasting properties as in NFL samples. As amylose contents of NFL-F samples compared to NFL samples were not significantly different except for samples NFL001F and NFL002F, no clear conclusion can be drawn about the effect of air classification on amylose content.

Hüttner et al. (2010) concluded that to produce a high quality oat bread, the whole oat flour should have low dough viscosity, low flour water hydration capacity, starch content of more than 65%, protein content of 12%, low damaged starch content and coarse particle size. In the light of their results, it would be interesting to compare the baking performance of the NFL and NFL-F samples. NFL-F samples had lower damaged starch content and higher starch content than NFL samples, which could be favourable regarding the baking quality. However, small particle size of the endosperm fraction (NFL-F) may increase the flour hydration capacity and thus be unfavourable to the baking quality.

In conclusion, the separation of the bran and endosperm fractions of the oat flours was successful as the mass yields were in the aimed range and the efficient enrichment of starch into the fine fraction was obtained. In addition, the fractionation affected the pasting properties of the samples as the fine fractions exhibited different pasting behaviour than the original oat flours.

2.3.4 Limitations of the study

As mentioned previously, different macromolecules in oats can interfere in analytical methods used for analyzing the chemical properties of oats. The methods used in this thesis had good repeatability and accuracy and the performance of the methods was followed with known reference materials. Nevertheless, possible interferences caused by the sample components cannot be ruled out.

The Megazyme methods used in current study are suitable for both pure starch and wholegrain samples (Megazyme 2018c; Megazyme 2018b; Megazyme 2019). The reference materials in these methods were either pure maize starch or wheat flour. Many analytical methods have been developed mainly with other cereals than oats (Gibson et al. 1992; McCleary et al. 2012). Therefore, it could be expected that there are some challenges deriving from the unique characteristics of oats. For example, the filtration step described in the original Megazyme amylose content method was replaced with centrifugation in the current study as the oat samples clogged up the filters. Based on the results obtained for the reference maize starch, use of centrifugation did not affect the results.

In the current study, there were also some difficulties with the repeatability of the RVA results in some of the samples. Especially the NFL-F samples had more variation in the results. It seemed that some flour lumps remained in the sample suspensions despite the thorough mixing prior and in the beginning of the RVA analysis, which caused irregularities in the viscosities. The challenges were sample related as some of the samples exhibited consistently higher variation in the results than the other samples.

Possible further studies could include the determination of the dietary fibre content and amylose content of G samples in order to understand better the changes in the pasting properties caused by oat milling process. Furthermore, the total dietary fibre content of the coarse fraction (NFL-C) obtained by air-classification could be analysed in order to understand the effect of the TDF content on the dry fractionation properties of the oat flour. In addition, the β-glucan content of the samples could provide more information about the role of β-glucan in the pasting properties.

3 CONCLUSIONS

Understanding the variation between different oat sample characteristics and the effect of oat milling process on oat flour properties will improve the applicability of oats in food products. In the current study it was observed, that the ten different oat samples differed in their carbohydrate properties and behaved differently when processed with traditional oat milling process and dry fractionation when compared to non-processed samples.

The dietary fibre contents, damaged starch contents and amylose contents were in agreement with the previous literature. Furthermore, it was observed that milling process changes the pasting properties of the oat samples. Significantly higher peak viscosity, through viscosity, final viscosity and setback viscosity values were observed in industrially produced oat flours compared to the non-heat-treated oat groats. Oat milling process caused significant increase in the amount of starch damage, which seemed to affect the physicochemical properties of the oat raw materials. The amount of damaged starch was clearly sample dependent. Furthermore, the fine fraction obtained by air-classification exhibited significantly different pasting properties and carbohydrate quality compared to the original oat flour.

In the future studies it would be optimal to also obtain more information about the carbohydrate composition of the non-heat-treated sample, i.e. amylose content and dietary fibre content, to understand better the effects of oat milling on oat carbohydrate properties. The results obtained in this thesis indicate that the oat flours with varying carbohydrate

properties would also differ in their behaviour in the food processes. For example, it may be expected that the ten different oat flour samples would exhibit differing baking quality. Furthermore, it would be interesting to see, how different oat materials behave in oat drink or in yoghurt-type oat products.

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APPENDICES

APPENDIX 1. Pasting properties of the oat raw materials

Pasting properties of non-heat-treated oat groats (G), oat flour (NFL) and fine fractions (NFL-F) produced from oat flour with air classification from NFL flours with air classifier wheel speed of 2500 rpm and airflow of 220 m3/h in ten oat samples (codes 001-008,010 and 013). Values are as average values (n=3) ± standard deviation.

Sample	Peak viscosity (mPa*s)	Breakdown (mPa*s)	Final viscosity (mPa*s)	Setback (mPa*s)	Pasting temperature (°C)
G001	4128 ± 37	1751 ± 16	5489 ± 106	3112 ± 69	82.2 ± 0.4
NFL001	4281 ± 114	1904 ± 63	5758 ± 82	3381 ± 81	82.4 ± 0.9
NFL001F	4296 ± 13	2072 ± 15	5160 ± 147	2936 ± 130	84.5 ± 0.5
G002	3469 ± 131	1726 ± 79	4809 ± 94	3066 ± 38	86.5 ± 0.0
NFL002	4281 ± 114	1505 ± 118	7981 ± 923	5205 ± 956	74.6 ± 6.2
NFL002F	4236 ± 30	1912 ± 16	6733 ± 291	4408 ± 314	86.6 ± 0.0
G003	3816 ± 19	2014 ± 27	3913 ± 34	2111 ± 13	83.3 ± 0.0
NFL003	4372 ± 264	1714 ± 171	4825 ± 158	2167 ±63	79.5 ± 2.4
NFL003F	4890 ± 137	2260 ± 55	4880 ± 248	2250 ± 143	74.3 ± 3.6
G004	3787 ± 12	1878 ± 27	4223 ± 68	2314 ± 55	83.3 ± 0.0
NFL004	4548 ± 27	2172 ± 40	6318 ± 436	3942 ± 449	86.0 ± 0.9
NFL004F	4559 ± 116	2356 ± 83	4614 ± 288	2411 ± 301	86.6 ± 0.0
G005	3306 ± 34	1784 ± 25	4100 ± 119	2577 ± 116	85.2 ± 0.6
NFL005	4520 ± 233	1644 ± 157	6254 ± 111	3379 ± 27	85.2 ± 0.5
NFL005F	5069 ± 116	2200 ± 63	6267 ± 181	3398 ± 171	84.7 ± 1.3
G006	4736 ± 14	2441 ± 6	4414 ± 65	2119 ± 63	83.9 ± 0.5
NFL006	5674 ± 15	2596 ± 54	6379 ± 144	3301 ± 172	83.4 ± 0.0
NFL006F	5729 ± 93	2855 ± 43	5383 ± 240	2510 ± 180	84.1 ± 0.8
G007	3048 ± 58	1842 ± 22	3141 ± 64	1934 ± 32	84.4 ± 0.9
NFL007	4656 ± 45	2154 ± 5	4661 ± 36	2160 ± 13	83.3 ± 0.1
NFL007F	5116 ± 29	2569 ± 15	4573 ± 119	2025 ± 148	82.8 ± 0.5
G008	4118 ± 34	1969 ± 21	4529 ± 35	2380 ± 48	83.9 ± 0.4
NFL008	4969 ± 162	1792 ± 57	5771 ± 164	2594 ± 56	81.9 ± 0.5
NFL008F	5313 ± 17	2139 ± 17	5505 ± 17	2331 ± 27	81.4 ± 0.4
G010	3571 ± 22	1975 ± 7	3450 ± 36	1853 ± 18	80.8 ± 0.0
NFL010	4579 ± 307	2151 ± 187	4615 ± 171	2188 ± 85	82.8 ± 0.4
NFL010F	4986 ± 27	2621 ± 37	4131 ± 241	1766 ± 222	83.3 ± 0.0
G013	3481 ± 18	1919 ± 23	4659 ± 18	3098 ± 27	87.6 ± 0.4
NFL013	4256 ± 126	1602 ± 29	6872 ± 170	4217 ± 113	84.9 ± 0.8
NFL013F	4430 ± 62	1878 ± 26	6274 ± 244	3722 ± 241	86.3 ± 0.5